§ 113.317 Parvovirus Vaccine (Canine).

Parvovirus Vaccine recommended for use in dogs shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this section.

(b) The Master Seed shall be tested for reversion to virulence in dogs using a method acceptable to Animal and Plant Health Inspection Service. If a significant increase in virulence is seen within five backpassages, the Master Seed is unsatisfactory.

(c) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five canine parvovirus susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine parvovirus to determine susceptibility. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of canine parvovirus.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Fourteen days or more after the final dose of vaccine the vaccinates and the controls shall be challenged with virulent canine parvovirus furnished or approved by Animal and Plant Health Inspection Service and the dogs observed each day for 14 days. Rectal temperature, blood lymphocyte count, and feces for viral detection shall be taken from each dog each day for at least 10 days postchallenge and the presence or absence of clinical signs noted and recorded each day.

(i) The immunogenicity of the Master Seed shall be evaluated on the following criteria of infection: temperature ≥103.4 °F; lymphopenia of ≥50 percent of prechallenge normal; clinical signs such as diarrhea, mucus in feces, or blood in feces; and viral hemagglutinins at a level of ≥1:64 in a 1:5 dilution of feces or a test of equal sensitivity. If at least 80 percent of the controls do not show at least three of the four criteria of infection during the observation period, the test is a No Test and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing more than one criterion of infection described in paragraph (c)(3)(i), of this section, the Master Seed is unsatisfactory.

(4) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine used in the immunogenicity test in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the

immunogenicity test, but not less than $10^{2.5}$ ID_{50} per dose.

[50 FR 436, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§ 113.318 Pseudorabies Vaccine.

Pseudorabies Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five pseudorabies susceptible pigs (20 vaccinates and 5 controls) of the youngest age for which the vaccine is recommended, shall be used as test animals. Blood samples shall be taken from each pig and the serums inactivated and individually tested for neutralizing antibody against pseudorabies virus. Pigs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test using 50 to 300 $TCID_{50}$ pseudorabies virus.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 pigs used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) Fourteen to 28 days postvaccination, the vaccinates and controls shall be challenged with virulent pseudorabies virus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days.

(i) If at least four of the five controls do not develop severe central nervous system signs or die, the test is a No Test and may be repeated.

(ii) If at least 19 of the 20 vaccinates in a valid test do not remain free of signs of pseudorabies, the Master Seed is unsatisfactory.

(4) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph.

(2) *Virus titer requirements.* Final container samples of completed product shall be titrated by the method used in paragraph (b)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer at least $10.^{0.7}$ greater than that used in the immunogenicity test, but not less than $10^{2.5}$ $TCID_{50}$ per dose.

[50 FR 437, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§§ 113.319–113.324 [Reserved]

§ 113.325 Avian Encephalomyelitis Vaccine.

Avian Encephalomyelitis Vaccine shall be prepared from virus-bearing tissues or fluids from embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 and the requirements prescribed in this section.